AMENDMENTS

In the Specification:

Please amend the specification as follows:

At page 1, lines 8-9:

This Application claims the benefit of the U.S. Provisional Application by Barone *et al.* (USSN 60/003726) filed September 13, 1995.

At page 1, lines 19-30:

Methods of forming large arrays of oligonucleotides, peptides and other polymers on a solid substrate are known. Pirrung *et al.*, U.S. Patent No. 5,143,854 (see also PCT Application No. WO 90/15070), McGall *et al.*, USSN 06/440742, Chee *et al.*, SN PCT/US94/12305, and Fodor *et al.*, PCT Publication No. WO 92/10092 describe methods of forming vast arrays of peptides, oligonucleotides and other polymers using, for example, light-directed synthesis techniques.

In the Fodor *et al.* PCT application, methods are described for using computer-controlled systems to direct polymer array synthesis. Using the Fodor approach, one heterogeneous array of polymers is converted, through simultaneous coupling at multiple reaction sites, into a different heterogeneous array. *See also*, USSN 07/796,243 and USSN 07/980,523 and Fodor *et al.* (1991) *Science*, 251: 767-777.

At page 2, lines 3-20:

More recently, US applications USSN 06/440,742, USSN 08/284,064, USSN 08/143,312, USSN 08/082,937 and PCT application (designating the United States) SN PCT/U594/12305, describe methods of making arrays of oligonucleotide and oligonucleotide analogue probes, *e.g.*, to check or determine a partial or complete sequence of a target nucleic acid, or to detect the presence of a nucleic acid containing a specific oligonucleotide sequence. USSN application 08/327,687 and USSN application 06/440,742 describe methods of creating libraries of nucleic acid probes for the analysis of nucleic acid hybridization, and for screening nucleic acid binding molecules, *e.g.*, as potential therapeutic agents.

Additional methods applicable to polymer synthesis on a substrate are described in co-pending Applications USSN 07/980,523, filed November 20, 1992, and USSN 07/796,243, filed November 22, 1991, incorporated herein by reference for all purposes. In the methods disclosed in these applications, reagents are delivered to the substrate by flowing or spotting polymer synthesis reagents on predefined regions of the solid substrate. In each instance, certain activated regions of the substrate are physically separated from other regions when the monomer solutions are delivered to the various reaction sites, *e.g.*, by means of grooves, wells and the like.

At page 3, lines 9-15:

The present invention provides methods and compositions to monitor the synthesis and coupling of monomers and polymers to solid substrates, *e.g.*, in VLSIPS™ arrays. The methods typically operate by incorporating a detectable label (typically an isomeric label, *e.g.*, as provided by the compositions herein) into the polymers of an array. The polymers are cleaved

from the array, and the efficiency of polymer synthesis assessed by monitoring the detectable label in an appropriate assay.

At page 6, lines 13-21:

In another class of preferred embodiments, the invention provides an array of polymers, such as an array of oligonucleotides or proteins, or non-biological polymers, with a monoisomeric detectable label incorporated into each polymer. For instance, in one embodiment where the array is an oligonucleotide, the invention provides an array of oligonucleotides attached to a solid substrate, wherein the label is a monoisomeric label comprising the structure wherein F comprises a fluorescent group;

At page 7, lines 12-17:

In one preferred group of embodiments, the nucleic acid synthesis reagent has the structure

wherein R_1 is selected from the group consisting of alkyl, aryl, and hydrogen; R_2 is selected from the group consisting of alkyl, and aryl; and FL is a fluorescent moiety.

At page 9, lines 24-26:

R_1 is selected from the group consisting of hydrogen, alkyl and aryl;

R_2 is selected from the group consisting of hydrogen, alkyl and aryl;

R_3 is selected from the group consisting of hydrogen, alkyl and aryl;

The term "capping" in the context of synthesizing an array of polymers refers to a step in which unreacted groups that fail to condense and successfully couple with the next polymer synthesis reagent (*e.g.*, a monomer such as a phosphoramidite or amino acid) are blocked. This insures that subsequent reactions proceed only by propagating chains of desired sequence. For instance, capping typically involves the acetylation of 5'-hydroxyl functions on oligonucleotides. This is accomplished, *e.g.*, using acetic anhydride catalyzed by 4-dimethylaminopyridine (DMAP). Other reagents known to those of skill in the art are also suitable.

A "nucleic acid" is a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogs of natural nucleotides that function in a manner similar to naturally occurring nucleotides (*See*, copending application USSN 06/440742 for a description of nucleic acid analogues).

An "oligonucleotide" is a nucleic acid polymer composed of two or more nucleotides or nucleotide analogues. An oligonucleotide can be derived from natural sources but is often synthesized chemically. It is of any size. Copending application USSN 06/440742 describes a variety of oligonucleotide analogues.

support materials include, but are not limited to, glass, polacryloylmorpholide, silica, controlled pore glass (CPG), polystyrene, polystyrene/latex, and carboxyl modified teflon. The solid

substrates are biological, nonbiological, organic, inorganic, or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, *etc.* depending upon the particular application. In light-directed synthetic techniques, the solid substrate is often planar but optionally takes on alternative surface configurations. For example, the solid substrate optionally contains raised or depressed regions on which synthesis takes place. In some embodiments, the solid substrate is chosen to provide appropriate light-absorbing characteristics. For example, the substrate may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SiN_4, modified silicon, or any one of a variety of gels or polymers such as (poly)tetrafluoroethylene, (poly)vinylidendifluoride, polystyrene, polycarbonate, or combinations thereof. Other suitable solid substrate materials will be readily apparent to those of skill in the art. Preferably, the surface of the solid substrate will contain reactive groups, such as carboxyl, amino, hydroxyl, thiol, or the like. More preferably, the surface is optically transparent and has surface Si—OH functionalities, such as are found on silica surfaces. A substrate is a material having a rigid or semi-rigid surface. In spotting or flowing VLSIPS™ techniques, at least one surface of the solid substrate is optionally planar, although in many embodiments it is desirable to physically separate synthesis regions for different polymers with, for example, wells, raised regions, etched trenches, or the like. In some embodiments, the substrate itself contains wells, trenches, flow through regions, *etc.* which form all or part of the regions upon which polymer synthesis occurs.

At page 19, lines 20-27:

As described above, diverse methods of making polymer arrays are known; accordingly no attempt is made to describe or catalogue all known methods. For exemplary purposes, light directed VLSIPS™ methods are briefly described below. One of skill will understand that alternate methods of creating polymer arrays, such as spotting and/or flowing reagents over defined regions of a solid substrate, bead based methods and pin-based methods are also known and applicable to the present invention (*See*, Holmes *et al.* (filed January 17, 1995) USSN 08/374,492).

At page 20, lines 26-30:

As described above, several methods for the synthesis of polymer arrays are known. In preferred embodiments, the polymers are synthesized directly on a solid surface as described above. However, in certain embodiments, it is useful to synthesize the polymers and then couple the polymers to the solid substrate to form the desired array. In these embodiments, polymers are

At page 23, lines 1-9:

described by Beaucage *et al.* (Beaucage *et al.* (1981) *Tetrahedron Letts.* 22 (20): 1859-1862) prior to attachment on a solid substrate. Bead-based synthetic techniques are described in copending application USSN 07/762,522 (filed September 18, 1991); USSN 07/946,239 (filed September 16, 1992); USSN 08/146,886 (filed November 2, 1993); USSN 07/876, 792 (filed April 29, 1992); PCT/US93/04145 (filed April 28, 1993); and Holmes *et al.* (filed January 17, 1995) USSN 08/374,492. Finally, as described above, polymers are optionally synthesized using

VLSIPS™ methods in arrays, or optionally cleaved from the array and then reattached to a solid substrate to form a second array.

At page 24, lines 1-18:

optionally protected during polymer synthesis using protecting groups. Among a wide variety of protecting groups which are useful are nitroveratryl (NVOC) α-methylnitroveratryl (Menvoc), allyloxycarbonyl (ALLOC), fluorenylmethoxycarbonyl (FMOC), α-methylnitro-piperonyloxycarbonyl (MeNPOC), -NH-FMOC groups, t-butyl esters, t-butyl ethers, and the like as described, *e.g.*, by Holmes *et al.* (*id*). Various exemplary protecting groups are described in, for example, Atherton *et al.*, (1989) *Solid Phase Peptide Synthesis*, IRL Press, and Greene, *et al.* (1991) *Protective Groups In Organic Chemistry*, 2nd Ed., John Wiley & Sons, New York, NY. The proper selection of protecting groups for a particular synthesis is governed by the overall methods employed in the synthesis. For example, in "light-directed" synthesis, discussed herein, the protecting groups are photolabile protecting groups such as NVOC, MeNPoc, and those described in co-pending Application PCT/U593/10162 (filed October 22, 1993). *See also,* Holmes *et al.* (*supra*); Wang (1976) *J. Org. Chem.* 41: 3258; and Rich, *et al.* (1975) *J. Am. Chem. Soc.* 97: 1575-1579. In other methods, protecting groups are removed chemically, and include groups such as FMOC, di(*p*-methoxyphenyl)phenyl (DMT) and others known to those of skill in the art. *See,* Holmes *et al.* (*supra*).

At page 25, lines 12-29:

In preferred embodiments, the present invention proceeds by labeling polymers in arrays, which are then cleaved from the arrays and analyzed. A variety of labels are appropriate and known. A "label" comprises a moiety which is detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (*e.g.*, as commonly used in an ELISA), biotin, dioxigenin, haptens and proteins. In preferred embodiments, the label is detectable spectroscopically, *i.e.*, is chromogenic. Suitable chromogens include molecules and compounds which absorb light in a distinctive range of wavelengths so that a color may be observed, or emit light when irradiated with radiation of a particular wavelength or wavelength range (*e.g.*, a fluorescent label). In preferred embodiments, labels of the present invention have the structure A—B, where A is a detectable moiety, and B is a "linking" or "bridging" group which comprises one or more functional regions which allow the detectable moiety to be incorporated into a polymer, or attached to one end of the polymer, using chemistry similar to that used to connect monomers into the polymer. Examples of suitable bridging regions include alkyl and substituted alkyl carbon chains with 1-30 carbons, or more preferably 3-10 carbons, with functional groups such as oxygen and

At page 28, lines 1-12:

nucleic acid integration element comprising a phosphorous atom; Y is selected from the group consisting of hydrogen, alkyl, or aryl; Y_2 is an alkyl chain from 1 to 30 carbons in length; Z comprises a protecting group; and F comprises a fluorescent group.

In a still more preferred embodiment, the nucleic acid synthesis reagent has the structure

wherein R_1 is selected from the group consisting of alkyl, aryl, and hydrogen; R_2 is selected from the group consisting of hydrogen, alkyl and aryl; and FL is a fluorescent moiety. An example of such a nucleic acid synthesis reagent label is the isomeric nucleic acid synthesis reagent with the structure

At page 30, lines 1-29:

oxacarbocyanine, merocyanine, 3-aminoequilenin, perylene, *bis*-benzoxazole, *bis*-p-oxazolyl benzene, 1,2-benzophenazin, retinol, *bis*-3-aminopyridiium salts, hellebrigenin, tetracycline, sterophenol, benzimidazolylphenylamine, 2-oxo-3-chromen, indole, xanthen, 7-hydroxycoumarin, phenoxazine, calicylate, strophanthidin, porphyrins, triarylmethanes and flavin. Individual fluorescent compounds which have functionalities for linking or which can be modified to incorporate such functionalities include, *e.g.*, dansyl .chloride; fluoresceins such as 3,6-dihydroxy-9-phenylxanthhydrol; rhodamineisothiocyanate; N-phenyl 1-amino-8-sulfonatonaphthalene; N-phenyl 2-amino-6-sulfonatonaphthalene; 4-acetamido-4-isothiocyanatostilbene2,2'-disulfonic acid; pyrene-3-sulfonic acid; 2-toluidinonaphthalene-6sulfonate; N-phenyl-N-methyl-2-aminoaphthalene-6-sulfonate; ethidium bromide; stebrine; auromine-0,2-(9'-anthroyl)palmitate; dansyl phosphatidylethanolamine; N,N'-dioctadecyl oxacarbocyanine; NN,N'-dihexyl oxacarbocyanine; merocyanine, 4-(3'pyrenyl)stearate; d-3-aminodesoxy-equilenin; 12-(9'-anthroyl)stearate; 2-methylanthracene; 9-vinylanthracene; 2,2'(vinylene-*p*-phenylene)bisbenzoxazole; *p*-*bis*(2-(4-methyl-5-phenyl-oxazolyl))benzene; 6-dimethylamino-1,2-benzophenazin; retinol; bis(3'-aminopyridinium) 1,10-decandiyl diiodide; sulfonaphthylhydrazone of hellibrienin; chlorotetracycline; N-(7-dimethylamino-4-methyl-2-

oxo-3chromenyl)maleimide;   N-(p-(2-benzimidazolyl)-phenyl)maleimide;   N-(4-fluoranthyl)maleimide;   bis(homovanillic   acid);   resazarin;   4-chloro-7-nitro-2,1,3-benzooxadiazole; merocyanine 540; resorufin; rose bengal; and 2,4-diphenyl-3(2H)-furanone. Many fluorescent tags are commercially available from SIGMA chemical company (Saint Louis, MO), Molecular Probes, R&D systems (Minneapolis, MN), Pharmacia LKB Biotechnology (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, WI), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersberg, MD), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), and

_2_ At page 31, lines 11-29:

Fluorescent labels are generally preferred, in part because by irradiating a fluorescent label with light, one can obtain a plurality of emissions. Thus, a single label can provide for a plurality of measurable events. Detectable signal may also be provided by chemiluminescent and bioluminescent sources. Chemiluminescent sources include a compound which becomes electronically excited by a chemical reaction and may then emit light which serves as the detectible signal or donates energy to a fluorescent acceptor. A diverse number of families of compounds have been found to provide chemiluminescence under a variety or conditions. One family of compounds is 2,3-dihydro-1,4-phthalazinedione. The most popular compound is luminol, which is the 5-amino compound. Other members of the family include the 5-amino-6,7,8-trimethoxy- and the dimethylamino[ca]benz analog. These compounds can be made to luminesce with alkaline hydrogen peroxide or calcium hypochlorite and base. Another family of

compounds is the 2,4,5-triphenylimidazoles, with lophine as the common name for the parent product. Chemiluminescent analogs include *para*-dimethylamino and -methoxy substituents. Chemiluminescence may also be obtained with oxalates, usually oxalyl active esters, *e.g.*, *p*-nitrophenyl and a peroxide, *e.g.*, hydrogen peroxide, under basic conditions. Other useful chemiluminescent compounds are also known and available, including -N-alkyl

At page 32, lines 21-30:

Accordingly, a preferred embodiment of the present invention utilizes a single optical isomer of all the possible diastereomers of a particular molecule as a label. Methods of purifying diastereomers, and methods of purifying enantiomers used to make diastereomers (i.e., "asymmetric synthesis") are known in the art. March (1992) *Advanced Organic Chemistry: Reactions, Mechanisms and Structure* Fourth Edition, John Wiley and Sons and the references therein, particularly chapter 4, and Lide (ed) *CRC Handbook of Chemistry and Physics* 75th edition and the references therein provide a general guide for the purification of stereoisomers. Briefly, a pair of enantiomers can be separated by reaction with a stereoselective reagent, reactions in the presence of circularly polarized light, or,

At page 38, lines 5-13:

Polymers cleaved from VLSIPS arrays are purified according to a variety of known techniques, including, but not limited to, gel electrophoresis, column chromatography, immunopurification, precipitation, crystallization, dialysis, filtration, high pressure liquid chromatography (HPLC), flash chromatography, paper chromatography and affinity

chromatography. *See, e.g.*, Sambrook, *supra*; Ausubel, *supra*; R. Scopes, *Protein Purification*, Springer-Verlag, N.Y. (1982); Hochuli (1989) *Chemische Industrie* 12:69-70; Hochuli (1990) *"Purification of recombinant proteins with Metal Chelate Absorbent"* in Setlow (ed.) *Genetic Engineering, Principle and Methods* 12:87-98, Plenum Press, N.Y.;

At page 44, lines 11-22:

To 15 g (88.4 mmol, 1 eq) of L-threonine methyl ester hydrochloride in 100 mL of dry THF at 0 °C under argon was added, dropwise over 1 hr, 265.3 mL (265.3 mmol, 3 eq) of borane-THF (1 M). The ice bath was removed and the reaction was stirred at room temperature overnight (18 hr). The solution was cooled to 0 °C and quenched slowly with 180 mL of 10 % acetic acid in methanol. The solution was then evaporated to a brown viscous oil and the oil co-evaporated three times with 100 mL of methanol. The crude material was purified by flash chromatography on silica gel using a step gradient of 1 % to 5 % conc. Ammonium hydroxide in methanol/dichloromethane 3:7 to afford 7.5 g (81 %) of (2S,3R)-2-amino-1,3-butanediol as a viscous oil. This material was dissolved in 50 mL of dry DMF and precipitated with hexanes in the cold to give 1 as a white solid.

At page 45, lines 3-13:

To 5 g (15.4 mmol, 1 eq) of N-Fmoc-4-aminobutyric acid and 8 mL (46.1 mmol, 3 eq) of dry diisopropylethylamine in 60 mL of dry THF at 0 °C under argon was added 2 mL (16.1 mmol, 1.05 eq) of pivaloyl chloride. The solution was stirred for 1 hr at 0 °C and then 1.8 g (16.9 mmol, 1.1 eq) of 1 was added in 8 mL of dry DMF. The solution was allowed to warm to

room temperature and the solvent removed under vacuum. The oil was dissolved in 100 mL of ethyl acetate and washed with 100 mL of sat. aq. NaHCO₃ and 100 mL of brine and dried over anhydrous Na₂SO₄. Filtration and solvent removal gave 7 g of an oil. The crude product was purified by flash chromatography on silica gel using ethyl acetate/hexanes/1 % triethylamine as eluent to afford 3.4 g (53 %) of 2 as a yellow foam.

At page 45, lines 16-24:

To 3.4 g (8.2 mmol, 1 eq) of 2 in 30 mL of dry pyridine under argon at ambient temperature was added 3.1 g (9.1 mmol, 1.1 eq) of 4,-4'-dimethoxytrityl chloride. The reaction was stirred for 18 hr and then the solvent removed under vacuum. The oil was taken up in 50 mL of ethyl acetate and washed twice with 50 mL of saturated aqueous NaHCO₃ and 50 mL of brine and dried over anhydrous Na₂SO₄. Filtration and removal of solvent gave about 7 g of an orange oil. The crude product was purified by flash chromatography on silica gel using ethyl acetate/hexanes 3:2 and 1 % triethylamine as eluent to afford 4.7 g (80 %) of 3 as a white foam.

At page 46, lines 2-10:

The FMOC group was removed by treatment of 4.7 g (6.6 mmol) of 3 with 50 mL of 20 % piperidine in DMF for 2 hr at ambient temperature. The solvent was removed under vacuum to give a white solid. The solid was dissolved in 200 mL of ethyl acetate and washed twice with 100 mL of saturated aqueous NaHCO₃ and 100 mL of brine and dried over anhydrous Na₂SO₄. Filtration and evaporation of the solvent gave a white solid which was purified through a plug of

silica gel using ethyl acetate/1 % triethylamine to remove the fulvene followed by elution with 60 % methanol/ethyl acetate/1 % triethylamine to afford 2.5 g (89 %) of 4 as a white foam.

At page 46, lines 13-27:

4 g (10.6 mmol, 1 eq) of 5-carboxyfluorescein was co-evaporated twice under vacuum with 30 mL of dry pyridine and the mixed with 1.2 g (10.6 mmol, 1 eq) of N-hydroxysuccinimide and 2.3 g (10.6 mmol, 1 eq) of dicyclohexylcarbodiimide in 100 mL of dry THF under argon. The reaction was stirred at ambient temperature for 18 hr and then filtered to remove the insoluble urea. The solvent was removed under vacuum to afford 5 g of an orange solid. To the crude NHS-ester in 50 mL of 10 % pyridine in dichloromethane was added amine 4 in 20 mL of dichloromethane under argon. The reaction was stirred overnight (18 hr) at ambient temperature. The reaction was poured into 100 mL of brine and the aqueous layer was extracted twice with 100 mL of dichloromethane/isopropyl alcohol 1:1. the organic fractions were combined and dried over anhydrous Na_2SO_4. Filtration and removal of solvent gave an orange solid which was purified by flash chromatography on silica gel using a stepwise gradient of 5 % to 30 % methanol/dichloromethane to afford 7.5 g (83 %) of 5 as a yellow solid.

At page 46, line 30 to page 47, line 8:

To 7.5 g (8.2 mmol, 1 eq) of 5 in 30 mL of dry dichloromethane under argon at ambient temperature was added 12.3 mL (16.4 mmol, 2 eq) of triethylamine and 0.2 g (1.6 mmol, 0.2 eq) of dimethylaminopyridine followed by 6.4 mL (16.4 mmol, 2 eq) of pivaloic anhydride. The reaction was stirred for 20 hr and washed twice with 100 mL of dilute aqueous $NaHCO_3$ (1/10

from saturation) and 100 mL of brine dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvent under vacuum gave a pale yellow foam which was purified by flash chromatography on silica gel using a methanol/dichloromethane/ethyl acetate mixture to afford 5.5 g (66 %) of <u>6</u> as a white solid.

At page 48, lines 8-15:

The DMT-on poly-16mer was cleaved automatically from the CPG support on the synthesizer with 2 mL of conc. NH_4OH into the collection vial containing 50 mL of 1 M NaOH (final concentration of NaOH is 25 mM), and allowed to stand in the dark at room temperature for 15 hr. The solution volume was reduced to about 0.5 mL in a speed-vac. The concentration of the oligonucleotide in A_{495} units (au) per mL was determined by dilution of the crude solution to obtain an absorbance reading between 0.1 au and 1 au/mL. The solution was stored at –20 °C in the dark.

At page 50, lines 1-5:

sealed tightly with a screw-cap and allowed to stand at room temperature for a minimum of 15 hrs. The solution was transferred with a pipetman to a 6 mL glass culture tube and the bottle/chip rinsed twice with 1 mL of $sdiH_2O$ and the rinse portions were added to the tube. The solution was evaporated to dryness in a speed-vac at medium heat and the residue suspended in 1 mL of $sdiH_2O$ (2 x 0.5 mL rinses).